

Figure 1

Hybridization Information

Module 1

Top Strand/Bottom Strand Types

DNA/DNA ☐

Top Strand Sequence 5'-3'

ccccccccccccccg

Bottom Strand Sequence 5'-3' ☐

Use Complement

ggtttttttttttgg

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ☐

[Monovalent cation] 0.105 mol/L

[Mg²⁺] 0 mol/L

Hybridization Temperature 37.0 °C

[Top Strand] 5e-8 mol/L

[Bottom Strand] 3e-7 mol/L

Corrections

Linear Correction for Micro Chips

$(\Delta G^{\circ}_{37}(\text{microchip}) = a \times \Delta G^{\circ}_{37}(\text{solution}) + b)$

a = 1 b = 0

Top Strand Folding Correction

$\Delta G^{\circ}_{37} = -2.1$ kcal/mol $\Delta H^{\circ} = -37.8$ kcal/m

Bottom Strand Folding Correction

$\Delta G^{\circ}_{37} = 0$ kcal/mol $\Delta H^{\circ} = 0$ kcal/m

Predict Thermodynamics

Clear Input

Figure 2a

3/16

Module 1

Duplex sequence

5'-CCCCAAAAAAAAAACCG-3'

3'-*GGTTTTTTTTTTTGG*-5'

Experimental conditions

Hybridization type = DNA/DNA

[Top strand] = 0.5E-07 mol/L

[Bottom strand] = 0.3E-06 mol/L

Hybridization temperature = 37.0 °C

Corrections

Top strand folding:

$\Delta H^{\circ} = -37.8$ kcal/mol

$\Delta G^{\circ}_{37.0} = -2.10$ kcal/mol

Thermodynamic predictions

In 1.000 M NaCl:

$\Delta H^{\circ} = -119.3$ kcal/mol

$\Delta S^{\circ} = -335.8$ eu

$\Delta G^{\circ}_{37.0} = -15.14$ kcal/mol

$T_M = 52.9$ °C

In 0.1050 M NaCl and 0.0000 M
MgCl₂:

$\Delta H^{\circ} = -119.3$ kcal/mol

$\Delta S^{\circ} = -348.3$ eu

$\Delta G^{\circ}_{37.0} = -11.29$ kcal/mol

$T_M = 42.2$ °C

The net hybridization thermodynamics
is:

$\Delta G^{\circ}_{37.0} = -8.74$ kcal/mol

$T_M = 34.9$ °C

Note:

The net hybridization temperature is the temperature at which the concentration of duplex equals half the maximum possible concentration of duplex.

The net free energy is calculated from the net equilibrium constant at the given temperature:

$K_{net} = \frac{[Duplex]}{([Ct - [Duplex]] * [Cb - [Duplex]])}$, where [Duplex] is the concentration of duplex, Ct is the initial concentration of top strand, Cb is the initial concentration of bottom strand.

Figure 2b

Hybridization Information

Module 2

Target/Primer Types

DNA/DNA ☐

Target Sequence 5'-3'



Primer length

15

Number of best primers to be displayed

2



Hybridization Conditions

User defined values for [Na+] and [Mg2+] ☐

[Monovalent cation] 1 mol/L

[Mg²⁺] 0 mol/L

Hybridization
Temperature 37.0 °C

[Target] 1e-6 mol/L

[Primer] 1e-6 mol/L

Corrections

Linear Correction for Micro Chips

$(\Delta G^{\circ}_{37}(\text{microchip}) = a \times \Delta G^{\circ}_{37}(\text{solution}) + b)$

a = 1 b = 0

Predict Primers

Clear Input

Figure 3a

5/16

Target sequence

5'-ACCGTTTGTA GTCCGTACGA CACATAACGG TGCATTG

Experimental conditions

Corrections

Hybridization type = DNA/DNA

No corrections

[Top strand] = 0.1E-05 mol/L

[Bottom strand] = 0.1E-05 mol/L

Hybridization temperature = 37.0 °C

[Na⁺] = 1.0000 mol/L

[Mg²⁺] = 0.0000 mol/L

The 2 best primers of length 15 are:

From position 28 to 42: 5'-GGTTGCAATGCACCG -3'

$\Delta H^{\circ} = -132.0$ kcal/mol $\Delta S^{\circ} = -355.6$ eu $\Delta G^{\circ}_{37.0} = -21.71$ kcal/mol $T_M = 70.2$ °C

From position 35 to 49: 5'-GCAGCATGGTTGCAA -3'

$\Delta H^{\circ} = -124.8$ kcal/mol $\Delta S^{\circ} = -336.5$ eu $\Delta G^{\circ}_{37.0} = -20.42$ kcal/mol $T_M = 68.4$ °C

Figure 3b

Hybridization Information

Target/Primer type

DNA/DNA

Target 5'-3'

acgcttgaatgcagttaatgcc

Primer Sequence 5'-3'

tgaatgcagt

Minimum percent stability of alternative binding sites
compared to the most stable binding site

50

Number of base pairs required to compute the solution

5

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ☐

[Monovalent cation] 1 mol/L

[Mg²⁺] 0 mol/L

Hybridization
Temperature 37.0 °C

[Target] 1e-6 mol/L

[Primer] 1e-6 mol/L

Corrections

Linear Correction for Micro Chips

$$(\Delta G^{\circ}_{37}(\text{microchip}) = a \times \Delta G^{\circ}_{37}(\text{solution}) + b)$$

a = 1 b = 0

Submit

Clear Input

Figure 4a

Figure 4b

Hybridization Information

Target/Primer Types

DNA/DNA ☒

Target Sequence 5'-3'

☒ Find best primer in sequence section ranging from
nucleotide number:

1

to

10

Primer length

15

☒ Number of best primer

1

Percent stability of alternative binding sites compared to the most stable binding site

50

Number of base pairs required to compute the solution

7

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ☒

[Monovalent cation] 1 mol/L

[Mg²⁺] 0 mol/L

Hybridization
Temperature 37.0 °C

[Target] 1e-6 mol/L

[Primer] 1e-6 mol/L

Corrections

Linear Correction for Micro Chips

$(\Delta G^{\circ}_{37}(\text{microchip}) = a \times \Delta G^{\circ}_{37}(\text{solution}) + b)$

a =

1

b =

0

Submit

Clear Input

Figure 5a

9/16

Target sequence

Module 5

5'-
AGGTCCATGCTTTGGAACAGCTACTTGAACCGATCATGGACACTGACGGATAACG
-3'

Experimental conditions

Corrections

Hybridization type = DNA/DNA

[Top strand] = 0.1E-05 mol/L

No corrections

[Bottom strand] = 0.1E-05 mol/L

Hybridization temperature = 37.0 °C

[Na⁺] = 1.0000 mol/L

[Mg²⁺] = 0.0000 mol/L

Number of base pairs required to compute the
solution = 7

Best primer search area from position 1 to
position 60

Best primer # 1:

from target position 35 to 49

5' -TCATGGACACTGACGGA-3'

3' -GTACCTGTGACTGCC-5'

$\Delta H^\circ = -123.1$ kcal/mol $\Delta S^\circ = -331.5$ eu $\Delta G^\circ_{37.0} = -20.27$ kcal/mol $T_M =$
68.4 °C

Best primer # 2:

from target position 18 to 32

5' -ACAGCTACTTGAACCGA-3'

3' -GTCCGATGAACTTGGC-5'

$\Delta H^\circ = -125.0$ kcal/mol $\Delta S^\circ = -339.6$ eu $\Delta G^\circ_{37.0} = -19.67$ kcal/mol $T_M =$
66.1 °C

Figure 5b

10/16

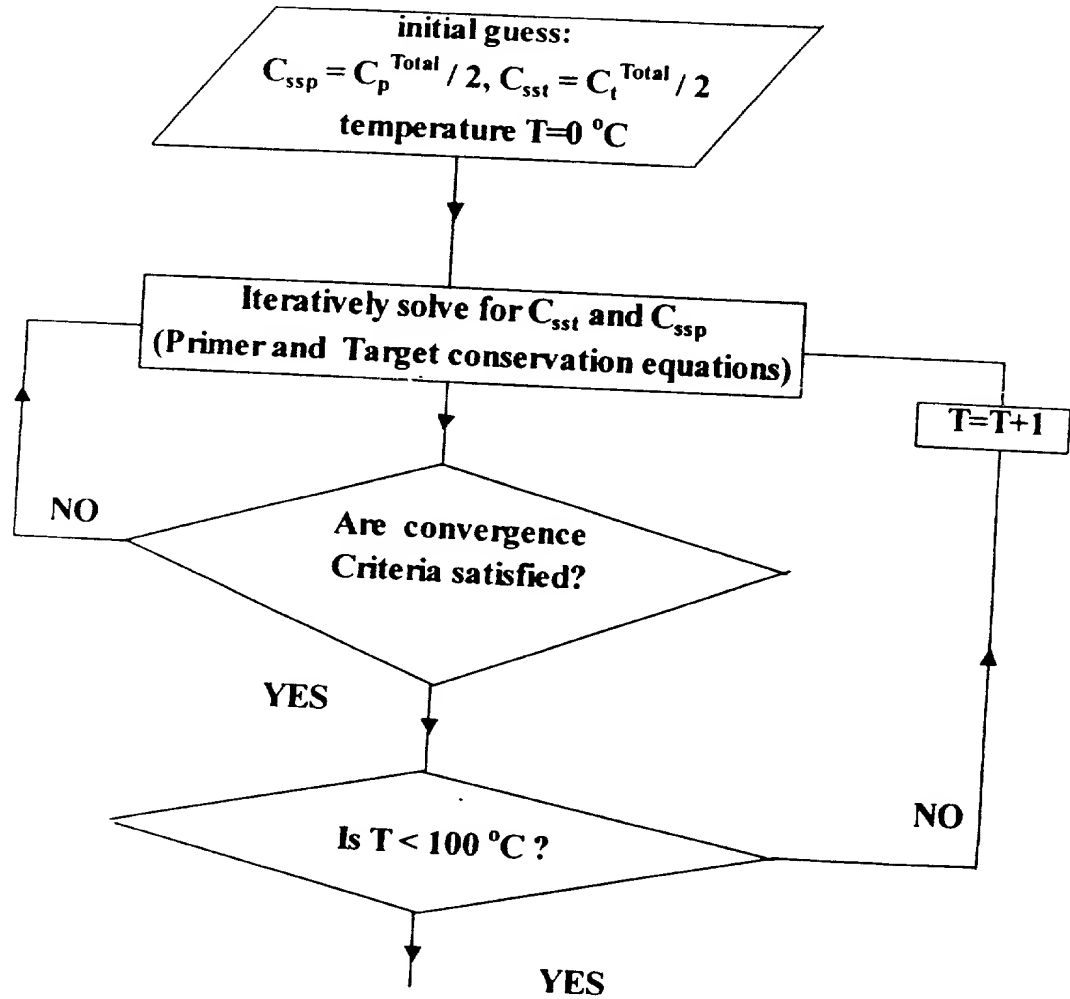


Figure 6

Multiplex PCR Design

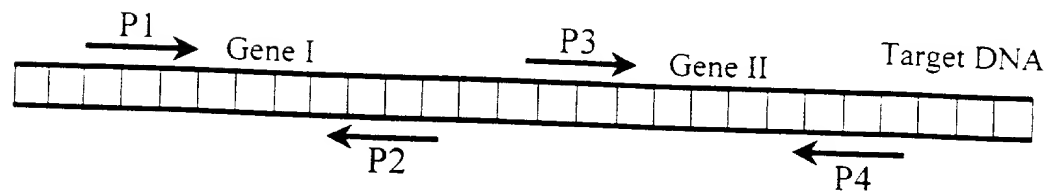
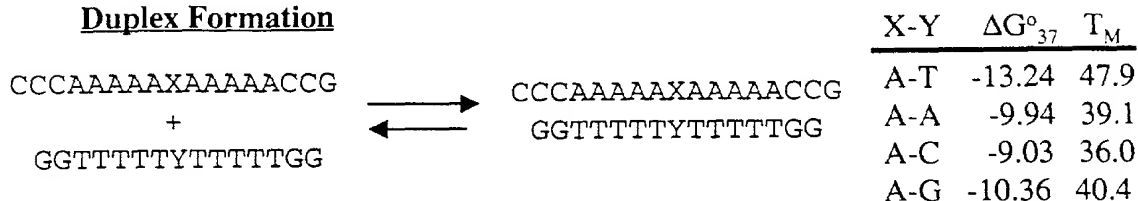


Figure 7

11/16

Prediction of Molecular Beacon Hybridization

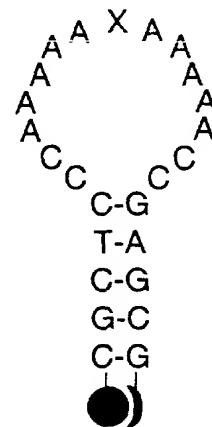
Duplex Formation



Beacon Folding

Random Coil Beacon

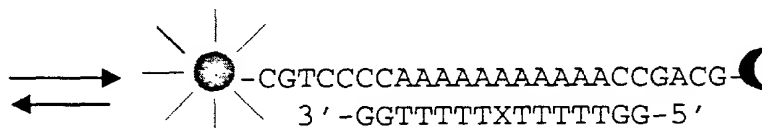
$$\Delta G_{37}^{\circ} = -2.1 \text{ kcal/mol} \quad T_M = 55.2^{\circ}\text{C}$$



Hairpin Beacon

Net Hybridization

Hairpin Beacon
+
Target



Target	X-Y	ΔG_{37}° (Effective)		T_M (Effective)	
		Exp.	Pred.	Exp.	Pred.
3'GGTTTTTTTTTTTGG5'	A-T	-10.49	-10.69	42	42.4
3'GGTTTTTATTTTGG5'	A-A	-6.66	-7.39	27	26.8
3'GGTTTTTCTTTTGG5'	A-C	-6.72	-6.48	23	21.1
3'GGTTTTTGTTTTGG5'	A-G	-7.62	-7.81	28	29.5

0.105 M NaCl 0.001 M MgCl₂ [beacon] = 5x10⁻⁸ M [target] = 3x10⁻⁷ M

Bonnet et al. (1999), *Proc. Nat. Acad. Sci. USA* 96, 6171-6176

Figure 8

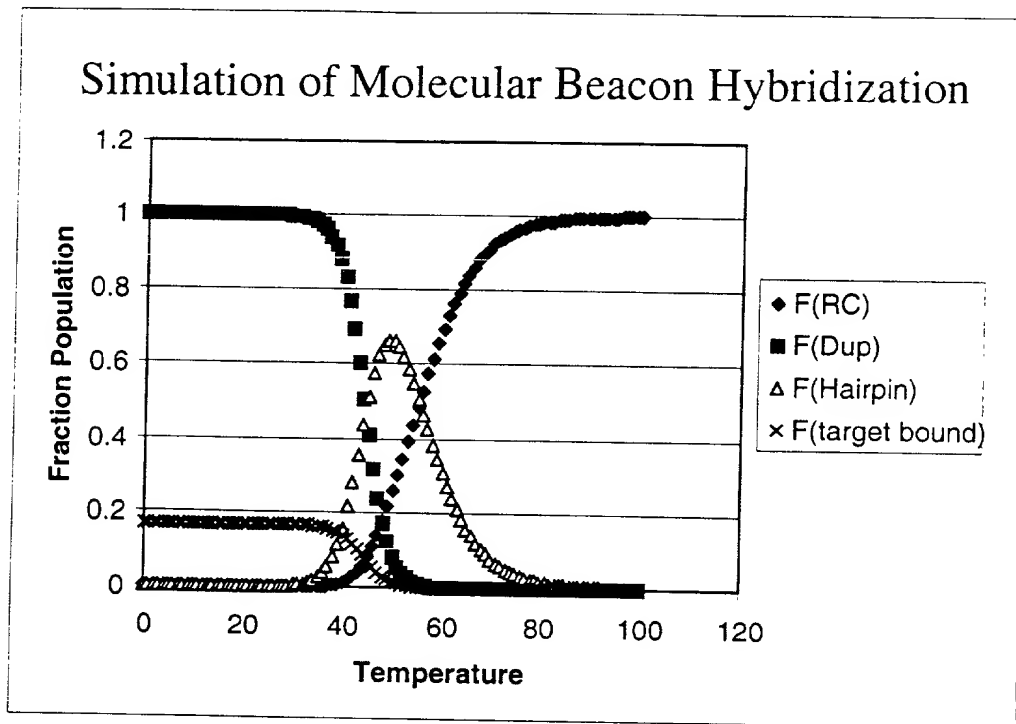
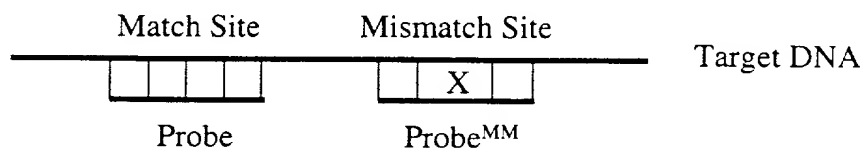
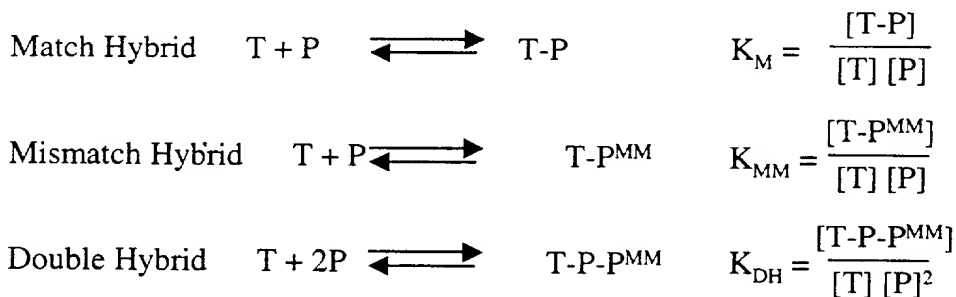


Figure 9

Match vs. Mismatch Hybridization



Equilibria



- Given $C_{\text{Target}}[\text{total}]$, $C_{\text{Probe}}[\text{total}]$ and the 3 equilibrium constants above, it is trivial to solve for the concentrations of all species
- Since ΔG_{37}° and ΔH° are known, calculate K 's at all temperatures
- Simulate hybridization at all temperatures - optimize specificity
- More complex model would also include single-strand folding equilibria

Figure10

Match vs. Mismatch Hybridization Simulation

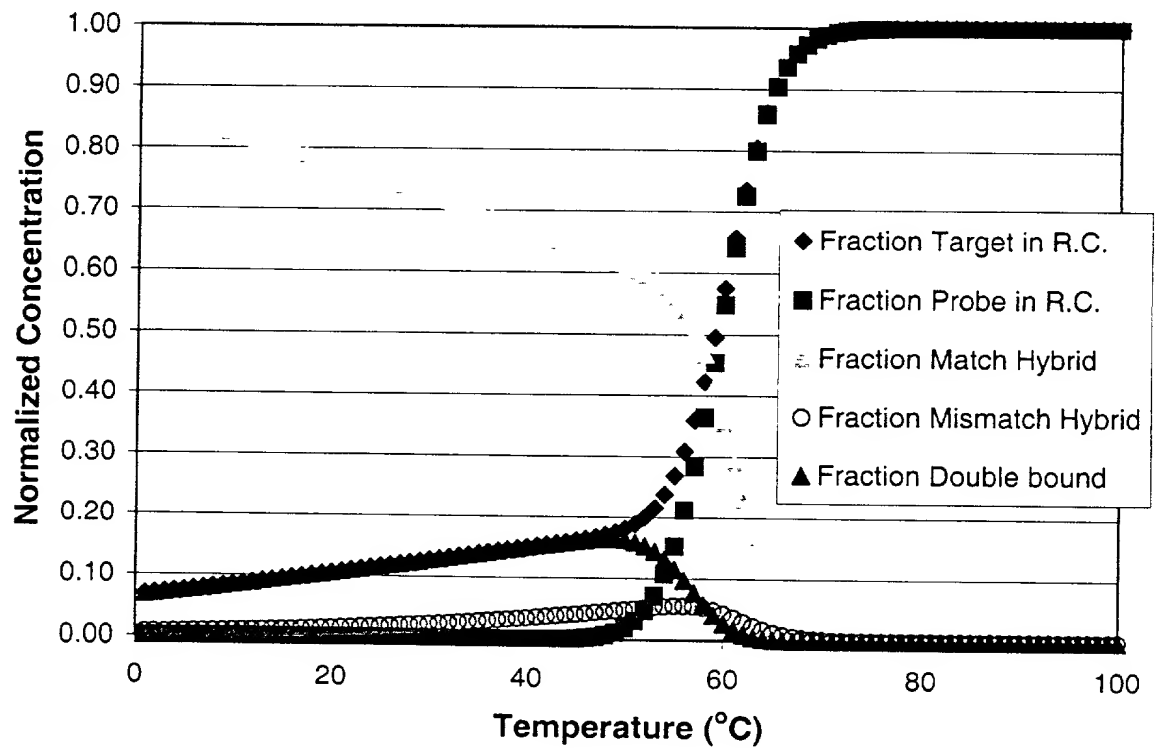


Figure11

SCANNED, #12

CONCENTRATION CALCULATIONS

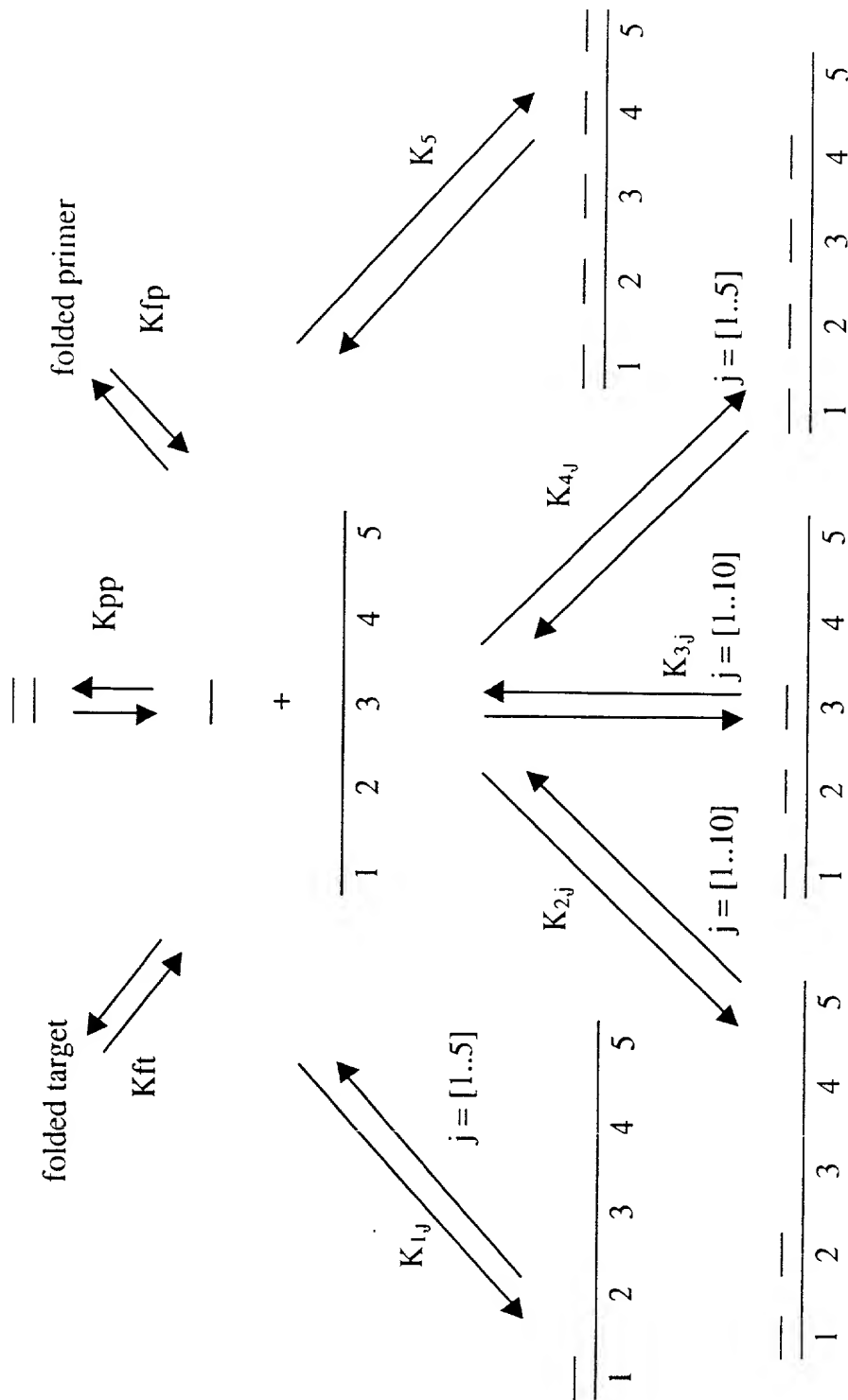


Figure12

PRIMER AND TARGET CONSERVATION EQUATIONS

$$\begin{aligned}
 C_{\text{sst}} = & C_{\text{t}}^{\text{Total}} / \{1 + K_{\text{ft}} + 2 \times K_{\text{tt}} \times C_{\text{sst}} \\
 & + C_{\text{ssp}} \times (K_1 + K_2 + K_3 + K_4 + K_5) \\
 & + C_{\text{ssp}}^2 \times (K_{1,2} + K_{1,3} + K_{1,4} + K_{1,5} + K_{2,3} + K_{2,4} + K_{2,5} + K_{3,4} + K_{3,5} + K_{4,5}) \\
 & + C_{\text{ssp}}^3 \times (K_{1,2,3} + K_{1,2,4} + K_{1,2,5} + K_{1,3,4} + K_{1,3,5} + K_{1,4,5} + K_{2,3,4} + K_{2,3,5} + K_{3,4,5} + K_{2,4,5}) \\
 & + C_{\text{ssp}}^4 \times (K_{1,2,3,4} + K_{1,2,3,5} + K_{2,3,4,5} + K_{1,3,4,5} + K_{1,2,4,5}) \\
 & + C_{\text{ssp}}^5 \times K_{1,2,3,4,5} \}
 \end{aligned}$$

$$\begin{aligned}
 C_{\text{ssp}} = & C_{\text{p}}^{\text{Total}} / (1 + K_{\text{fp}} + 2 \times K_{\text{pp}} \times C_{\text{ssp}} \\
 & + C_{\text{sst}} \times (K_1 + K_2 + K_3 + K_4 + K_5) \\
 & + 2 \times C_{\text{sst}} \times C_{\text{ssp}} \times (K_{1,2} + K_{1,3} + K_{1,4} + K_{1,5} + K_{2,3} + K_{2,4} + K_{2,5} + K_{3,4} + K_{3,5} + K_{4,5}) \\
 & + 3 \times C_{\text{sst}} \times C_{\text{ssp}}^2 \times (K_{1,2,3} + K_{1,2,4} + K_{1,2,5} + K_{1,3,4} + K_{1,3,5} + K_{1,4,5} + K_{2,3,4} + K_{2,3,5} \\
 & + K_{3,4,5} + K_{2,4,5}) \\
 & + 4 \times C_{\text{sst}} \times C_{\text{ssp}}^3 \times (K_{1,2,3,4} + K_{1,2,3,5} + K_{2,3,4,5} + K_{1,3,4,5} + K_{1,2,4,5}) \\
 & + 5 \times C_{\text{sst}} \times C_{\text{ssp}}^4 \times K_{1,2,3,4,5})
 \end{aligned}$$

Figure13